vascular conductance in all tissues. Results indicate that there are (1) a heterogeneous influence of nicotine in renal and splanchnic circulations associated with regional differences in activities of *alpha* and *beta* adrenergic receptors and (2) a potent non-adrenergic vasoconstrictor response in these circulations to nicotine after blockade of *alpha* and *beta* adrenergic receptors.

Downey, H. F., Crystal, G. J. and Bashour, F. A.

The Journal of Pharmacology and Experimental Therapeutics 220(2):375-381, 1982.

Other support: Cardiology Fund.

From the Departments of Physiology and Internal Medicine, University of Texas Health Science Center and Cardiovascular Research Laboratory at Methodist Hospital, Dallas.

EFFECT OF CIGARETTE SMOKING ON HIGH DENSITY LIPOPROTEIN PHOSPHOLIPIDS

This study was designed to assess the effect of acute inhalation of cigarette smoke on high density lipoprotein (HDL) phospholipid composition. In running the tests on White Carneau pigeons, the following four treatment regimens were instituted: (1) shelf control birds fed a chow diet and retained in their cages; (2) sham pigeons fed a cholesterol-saturated fat diet and exposed to fresh air by a smoking machine; (3) low nicotine-low carbon monoxide (LoLo) animals also fed the cholesterol-fat diet and exposed to low concentrations of these cigarette smoke products; and (4) high nicotine-high carbon monoxide (HiHi) birds fed the cholesterol-fat diet and subjected to high concentrations of these inhalants. Results of these studies showed that the cholesterol diet caused an increase in the concentration of most HDL phospholipid classes. Exposure to the HiHi regimen resulted in an increase in the HDL cholesterol/phospholipid ratio and a reduction in the concentration of HDL phosphatidyl ethanolamine, phosphatidyl serine/inositol, sphingomyelin and lysophosphatidyl choline. It appears, therefore, that cigarette smoking may attenuate HDL's anti-atherogenic properties by altering surface phospholipid components.

Hegarty, K. M., Turgiss, L. E., Mulligan, J. J., Cluette, J. E., Kew, R. R., Stack, D. J., and *Hojnacki, J. L.*

Biochemical and Biophysical Research Communications 104(1):212-219, 1982.

Other support: American Heart Association, Greater Boston Division.

From the Department of Biological Sciences, University of Lowell, Lowell, MA.

ALTERNATIVE COMPLEMENT PATHWAY-DEPENDENT INGESTION OF FLUOLITE PARTICLES BY HUMAN GRANULOCYTES

Particles of a fluorescent cyclic hydrocarbon (Fluolite) have been reported before to be ingested by mononuclear phagocytes of human blood. This paper describes

1002317073

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

quantitative studies of the ingestion of these same particles by human granulocytes. In the studies presented here, Fluolite particles with an average size of 0.1μM were ingested by human granulocytes after incubation in fresh normal human serum. Ingestion, which was assessed by visual counting in a fluorescent microscope of cells containing particles, required fresh normal serum and did not occur when serum was heated for 30 minutes at 50°C or when ethylenediaminetetraacetic acid (EDTA) was present. Particularly, it did not occur in serum genetically deficient in C3b inactivator or in C3. However, phagocytic activity was restored to C3-deficient serum by purified human C3 and to heat inactivated serum by purified factor B. Opsonization of particles under appropriate conditions is insensitive to the absence of human C2 or C5, but is dependent upon C3 and an intact alternative C pathway. Measurement of the opsonization of these particles thus constitutes a simple assay of the functional integrity of this pathway. Although the mechanism involved here is still unclear, this simple and readily available assay can be used clinically as a screen for the functional opsonic activity of the alternative C pathway, as well as for the capacity of human granulocytes to recognize and ingest C3-coated particles.

Arnaout, M. A., Luscinskas, F. W., *Lionetti, F. J.*, Alper, C. A., and Valeri, C. R.

The Journal of Immunology 127(1):278-281, 1981.

Other support: Office of Naval Research.

From the Division of Cell Biology and Nephrology, Children's Hospital Medical Center, Boston; Center for Blood Research, Boston; and Naval Blood Research Laboratory, Boston.

ENDOTHELIAL METABOLISM

There are three important functions of endothelium—(1) separation of blood and lymph from the extravascular space, (2) control of the influx and efflux of specific blood solutes and colloids, and (3) provision of a smooth gliding surface for passing blood—that have been well recognized for years. The book chapter presented here, though, deals not with these three functions but with the metabolic activities of the endothelium. While endothelial cells are well-equipped for glycolysis, oxidative phosphorylation and the complex series of reactions required for cell division, it is the number of far-reaching metabolic activities, most discovered since 1968, that are considered here. To wit, endothelial cells possess a highly complex array of intracellular machinery and can, in fact, elaborate molecules and macromolecules important not only to cell integrity and cell division but also important in terms of processing vasoactive and hemostatic substances. Many of the recently recognized metabolic activities occur at or near the cell surface. For example, angiotensin-converting enzyme appears to be disposed so that the enzyme itself is embedded in the plasma membrane, yet its catalytic site is situated to have access to angiotensin I and bradykinin as they pass in circulating blood. One section of this paper deals with anatomical considerations, and another with surveying the endothelium's known metabolic activities. While much of the work on endothelial metabolism performed so far has been accomplished through the disciplines of biochemistry, morphology, and cell biology,

10023170274

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

other studies, physiologic, pharmacologic, and pathologic, are feasible, and there is every reason to believe that clinically relevant data will be forthcoming.

Ryan, J. W. and *Ryan, U. S.*

In: Wiedman, M. P. (ed.): *An Introduction of Microcirculation*, New York: Academic Press, Inc. 1981, pp. 147-169.

Other support: U.S. Public Health Service and the John A. Hartford Foundation, Inc.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

STRUCTURAL BASES FOR METABOLIC ACTIVITY

The pulmonary endothelium, which is the first line of contact between blood-borne substrates and the cellular machinery of the lungs, is reviewed here along with those structural specializations that are suited for their role in the metabolic functions of the lungs. The underlying structural bases for metabolic activity are considered here under the three headings: anatomical, cellular, and subcellular. Particular emphasis is placed upon the enzymes, inhibitors, receptors, and transport mechanisms that are important in the regulation of the hormonal composition and fluidity of blood. Following anatomical study, a picture emerges of a variety of enzymes that are situated on the surface of pulmonary endothelial cells and are strategically poised for interaction with the appropriate substrates delivered by the blood and are equally strategically placed to determine the quantities of active substances allowed to pass downstream or into the extravascular space. Cellular studies show that the caveolae-plasma membrane fraction of lung homogenate converts angiotensin I to angiotensin II and degrades bradykinin to yield the characteristic products formed by intact lungs. Furthermore, the preparation can degrade ATP and 5′-AMP to adenosine and free phosphate. By use of a sensitive radioassay, it was also shown that pulmonary endothelial cells contain abundant angiotensin converting enzyme (ACE). In summary, it appears that the unique features of pulmonary enzymes may relate to the structure of the lungs and to the position of the lung in the circulatory system. Thus, the total amount of ACE within the lungs may greatly exceed the amount needed to process the concentration of angiotensin I and bradykinin usually found in pulmonary artery blood. While it is known that ACE occurs on the luminal surface of these cells, it is becoming evident at a subcellular level that the membrane bound enzyme is sensitive to factors such as oxygen concentration. However, the effects of components of the plasma membrane on the molecular configuration and hence on the activity of the enzyme are not known as yet.

Ryan, U. S.

Annual Review of Physiology 44:223-239, 1982.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

10023170Z5

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

NEUTROPHILS ARE REQUIRED FOR THE DNA SYNTHETIC RESPONSE OF HUMAN LYMPHOCYTES TO MEVALONIC ACID: EVIDENCE SUGGESTING THAT A NONSTEROL PRODUCT OF MEVALONATE IS INVOLVED

The ability of various enantiomeric forms of mevalonic acid to initiate lymphocyte DNA synthesis is examined in this paper, and the role of the neutrophilic polymorphonuclear leukocyte as the helper cell in evoking mevalonate-induced lymphocyte proliferation is defined with greater precision than has been accomplished before. The ability of lymphocytes to initiate DNA synthesis and cell cycling is a radiosensitive property of the cells, whereas the help provided by neutrophils is maintained despite their prior exposure to x-irradiation. Other organic acid anions, including precursors of mevalonic acid biosynthesis and a variety of products of mevalonate metabolism, fail to initiate DNA synthesis when added to human lymphocytes. Because only the metabolically active R(−) enantiomer of mevalonic acid initiates lymphocyte DNA synthesis, it seems probable that physiological pathways of mevalonate metabolism are involved. The response to mevalonic acid of ML-236B (compactin)-inhibited lymphocytes is increased, and the threshold concentration of mevalonate at which lymphocyte DNA synthesis first appears is decreased, when the cells are cultured in lipoprotein-containing medium. The response to mevalonic acid of lymphocytes cultured in lipoprotein-depleted medium can be enhanced by addition to the cultures of low density lipoprotein but not by addition of high density lipoprotein. Based upon the flux diversion hypothesis of mevalonate metabolism, these observations suggest that a nonsterol product of mevalonate metabolism may be responsible for the initiation of lymphocyte DNA synthesis by mevalonic acid.

Larson, R. A., Chung, J., Scanu, A. M., and *Yachnin, S.*

Proceedings of the National Academy of Sciences of the United States of America 79:3028-3032, 1982.

Other support: U.S. Public Health Service and the Nalco Center Research Fund.

From the Departments of Medicine and Biochemistry, the Franklin McLean Memorial Research Institute, and the Committee on Immunology, The University of Chicago School of Medicine, Chicago.

MEVALONIC ACID IN CONJUNCTION WITH HELP FROM NEUTROPHILS INDUCES DNA SYNTHESIS AND CELL CYCLING IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

Mevalonic acid plays an important role in the regulation of mammalian cell growth and division. Results from earlier studies have even suggested a critical role for mevalonic acid, independent of its conversion to cholesterol, in the regulation of DNA synthesis and cell replication. Evidence presented in this paper shows that mevalonic acid does stimulate DNA synthesis in human peripheral blood lymphocytes which have been isolated by gravity sedimentation of blood and freed of adherent cells by nylon column passage. Human peripheral blood mononuclear cells isolated by the Ficoll-Hypaque technique respond less well, but their response to mevalonic acid can be enhanced by the neutrophil-rich Ficoll-Hypaque-isolated "bottom" cell fraction. The kinetics of mevalonic acid-induced lymphocyte transformation are similar to those of more classic lymphocyte mitogens. In addition to stimulating lymphocyte DNA synthesis, mevalonic acid produces a population of cells representing all phases of the cell

10023176

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

cycle whose morphological characteristics are typical of those seen with more conventional mitogens. The DNA synthetic response of lymphocytes to mevalonic acid can be abolished by prior exposure of the lymphocytes to x-irradiation or mitomycin C, while the helper effect of granulocytes is unaffected by either treatment. These observations suggest that mevalonic acid not only may play a role as a critical substance which supports the propogation of cells programmed to divide, or stimulated to divide by various initiators of cell growth, but also that, in susceptible cell populations, mevalonic acid may act as an inducer of the entire program of the cell cycle.

Yachnin, S. and Richman, D. P.

Cellular Immunology 72:248-262, 1982.

Other support: U.S. Public Health Service, Muscular Dystrophy Association, and the Nalco Research Foundation.

From the Department of Medicine and Neurology, the Franklin McLean Memorial Research Institutes, and the Committee on Immunology, The University of Chicago School of Medicine, Chicago.

IV. *Neuropharmocology and Physiology*

NICOTINE BINDING SITES AND THEIR LOCALIZATION IN THE CENTRAL NERVOUS SYSTEM

The study of nicotine binding in the brain, which was plagued with problems during earlier investigations, has been helped greatly by the development of a method of obtaining the unnatural (+)-isomer optically pure and by the refined synthesis of radiolabeled nicotine with high specific activity. The actions of the stereoisomers of nicotine on the central nervous system are qualitatively similar in most tests but (−)-nicotine is more potent than the unnatural (+)-isomer by at least 10-fold. Binding of radiolabeled nicotine to brain has both saturable and nonsaturable components. Only saturable binding is affected by incubation conditions such as time, temperature, pH and ion concentration. Excess concentrations of the stereoisomers are equally effective in displacing (−)-[^{3}H]-nicotine from brain homogenates. Nevertheless, a direct comparison of (+)-[^{3}H]-nicotine and (−)-[^{3}H]-nicotine binding shows that the latter has a K_D three times lower than the former. (−)-[^{3}H]-nicotine is bound to the greatest degree in hypothalamus and hippocampus, areas that also exhibit the most stereoselectivity for nicotine. However, differences in the binding affinities of the two isomers are far less than the pharmacological stereospecificity observed.

Martin, B. R. and *Aceto, M. D.*

Neuroscience & Behavioral Reviews 3(4):473-478, 1981.

Other support: National Institutes of Health.

From the Department of Pharmacology, Medical College of Virginia, Richmond.

10023172077

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

CHARACTERIZATION OF NICOTINE BINDING IN MOUSE BRAIN AND COMPARISON WITH THE BINDING OF α-BUNGAROTOXIN AND QUINUCLIDINYL BENZILATE

The literature suggests that nicotine binds to neuronal tissue, but the nature and significance of this binding are in question. In this study of nicotine binding, the binding of [^{3}H]nicotine to mouse brain was measured and subsequently compared with the binding of [^{125}I]α-bungarotoxin (α-BTX) and L-[^{3}H] quinuclidinyl benzilate (QNB). The binding of nicotine was saturable, reversible and stereospecific. The average K_D and B_{max} were 59 nM and 88 fmoles/mg of protein, respectively. Although the rates of association and dissociation of nicotine were temperature-dependent, the incubation temperature had no effect on either K_D or B_{max}. When measured at 20° or 37°, nicotine appeared to bind to a single class of binding sites, but a second, very low-affinity, binding site was observed at 4°. Nicotine binding was unaffected by the addition of NaCl, KCl, $CaCl_2$, or $MgSO_4$ to the incubation medium. Nicotine cholinergic agonists were potent inhibitors of nicotine binding; however, nicotine antagonists were poor inhibitors. The regional distribution of binding was not uniform: midbrain and striatum contained the highest number of receptors, whereas cerebellum had the fewest. When nicotine, α-BTX, and QNB binding were compared in several ways, results indicated that all three ligands label cholinergic sites, but these sites differ from one another. Differences in site densities, regional distribution, inhibitor potencies, and thermal denaturation indicated that nicotine binding was not the same as either QNB or α-BTX binding, and therefore that receptors for nicotine may represent a unique population of cholinergic receptors.

Marks, M. J. and *Collins, A. C.*

Molecular Pharmacology 22:554-564, 1982.

Other support: National Institutes of Health.

From the Institute for Behavioral Genetics and School of Pharmacy, University of Colorado, Boulder.

EFFECTS OF ACUTE CENTRAL AND PERIPHERAL ADMINISTRATION OF NICOTINE ON HYPOTHALAMIC CATECHOLAMINE NERVE TERMINAL SYSTEMS AND ON THE SECRETION OF ADENOHYPOPHYSEAL HORMONES IN THE MALE RAT

The actions of intraventricular injections and intravenous infusions of nicotine were evaluated on dopamine and noradrenaline stores and turnover in discrete hypothalamic dopamine and noradrenaline nerve terminal systems in male Sprague-Dawley rats. Anterior pituitary hormone secretion was also studied in the same group of animals. Specifically, measurements were made of LH, FSH, GH, TSH, prolactin and corticosterone serum levels using radioimmunological procedures. Intraindividual correlations between regional catecholamine levels and turnover and hormone secretion were performed. Results showed that intraventricular injections of nicotine produced dose-dependent reductions of dopamine and noradrenaline levels and increases of dopamine and noradrenaline turnover in discrete hypothalamic areas. The turnover

1002317028

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

increases were associated with reductions of serum levels of TSH, prolactin and LH and an increase of serum FSH levels. Correlation analysis in the intraventricular experiments was performed. Also, intravenous infusions of nicotine over one hour produced dose-dependent reduction of dopamine and noradrenaline concentrations and increased the turnover of these monoamines in the various hypothalamic catecholamine nerve terminal systems analyzed, with the median eminence, dopamine and noradrenaline nerve terminal systems showing the highest sensitivity to nicotine. Overall, the total of these and other experimental findings reinforces the view that nicotine may act directly on the brain to activate dopamine and noradrenaline nerve terminal systems, probably via activation of nicotine-like cholinergic receptors. Correlation analysis shows that other neurotransmitter mechanisms must also be involved in producing the nicotine-induced changes in the secretion of anterior pituitary hormones.

Andersson, K., *Fuxe, K.*, Eneroth, P., and Agnati, L. F.

Medical Biology 60:98-111, 1982.

Other support: Svenska Tobaks AB, Stockholm.

From the Department of Histology, Karolinska Institutet, and the Hormone Laboratory, Karolinska Hospital, Stockholm: and the Department of Human Physiology, University of Modena, Modena, Italy.

ON THE INTERACTION BETWEEN NICOTINE AND CYCLOHEXIMIDE

Inhibitors of protein synthesis, such as cycloheximide, have been shown to affect memory retention and nicotine has been shown to reverse the amnestic properties of cycloheximide. In the study presented here, the interactions of nicotine and cycloheximide on brain protein synthesis were examined in an attempt to determine whether the nicotine-induced reversal of the amnestic effect of cycloheximide is due to an interaction between nicotine and cycloheximide on brain protein synthesis, and whether it involves the sites in brain that saturably bind [^{3}H]nicotine. Results showed that nicotine did not reverse the cycloheximide-induced inhibition of protein synthesis, both *in vivo* (intact animal) and *in vitro* (brain slices), suggesting that on-going protein synthesis is not necessarily involved in memory consolidation. Also, the nicotine binding sites were not affected by *in vivo* or *in vitro* treatment with cycloheximide either in the presence or absence of nicotine.

Sershen, H., Reith, M. E. A. and *Lajtha, A.*

Brain Research 251:183-185, 1982.

From the Center for Neurochemistry, Rockland Research Institute, Ward's Island, NY.

EVIDENCE FOR A NONCHOLINERGIC NICOTINE RECEPTOR ON HUMAN PHAGOCYTIC LEUKOCYTES

Radioligand binding studies show that leukocytes contain a number of peptide and hormone receptors, such as those for chemotactic peptides and acetylcholine. In this

1002317079

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

attempt to test the hypothesis that nicotine acts as a receptor (known or unknown), the specific binding of ^{3}H-(d,l) nicotine to leukocytes was measured. Results of this study demonstrate the presence of a noncholinergic nicotine receptor on human phagocytic leukocytes. The average affinity +/− standard deviation of (d,l)-nicotine for the receptor on neutrophils is 36 +/− 18 nM (n=6). The binding is saturable with an average of 8.7×10^4 sites per neutrophil. Monocytes and, to a lesser extent, lymphocytes but not erythrocytes also display specific binding. Bound nicotine is dissociable from the receptor and is not metabolized. Only close structural analogs of nicotine bind to the receptor, which is stereoselective for the (d)-isomer. The receptor can be occupied by (1)-nicotine at concentrations present in the blood of smokers. It is suggested that some of the adverse effects of smoking on leukocyte functions may be mediated by a specific nicotine receptor.

Davies, B. D., *Hoss, W.*, Lin, J-P., and Lionetti, F.

Molecular and Cellular Biochemistry 44:23-31, 1982.

Other support: U. S. Public Health Service.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY, and Center for Blood Research, Boston.

CHARACTERIZATION OF THE ISOLATED PERFUSED MOUSE BRAIN AS A SYSTEM FOR NEUROCHEMICAL STUDIES

The criteria for characterization of isolated perfused brain preparations include the demonstration of electrical, structural and metabolic viability. The purpose of this study was to meet these criteria and to establish the isolated perfused mouse brain (IPMB) as a viable model for neurochemical and neuropharmacological studies. In the paper presented here, the preparation of the IPMB is described along with its electrophysiological, morphological, biochemical and pharmacological properties. Using high performance liquid chromatography with electrochemical detection, the primary metabolite of mammalian central nervous system norepinephrine, 3-methoxy-4-hydroxyphenethyleneglycol (MHPG), was measured in the perfusate at 15-min. intervals. The rate of MHPG production was similar to literature values of the rate of norepinephrine turnover in mouse brain. MHPG production rate in the IPMB was blocked by pretreatment with 6-hydroxydopamine and was increased by pretreatment with reserpine.

Towell, J. F. and *Erwin, V. G.*

Brain Research 209:476-481, 1981.

Other support: U. S. Public Health Service and the University of Colorado National Center for Alcohol Research.

From the School of Pharmacy, University of Colorado, Boulder.

ANALYSIS OF REGIONAL VARIATIONS IN THE AFFINITIES OF MUSCARINIC AGONISTS IN THE RAT BRAIN

The specific binding of radiolabeled antagonists has been used to characterize the muscarinic acetylcholine receptor in brain. For the study reported here, the affinities of

1002317080

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

muscarinic agents in brains from male Sprague-Dawley rats were determined by direct and indirect assays of binding to the receptor. Results showed that the brain stem of the rat has a higher affinity toward muscarinic agonists than does the forebrain, Receptor occupancy curves of both regions of the brain deviate from simple mass-action binding. The characteristics of the binding in each region are compatible with the existence of two non-interacting binding sites, and are not attributable to desensitization or to negatively cooperative binding within a small oligomer; however, the possibility of large oligomers remains to be excluded. The agonist binding data were analyzed by a linear transformation of Scatchard-like inhibition curves of the binding of the antagonist [^{3}H]quinuclidinyl benzilate. Such analysis, based on model of two subpopulations of receptors in each area, shows the subpopulations of the brain stem and the forebrain to be distinct. Brain stem: 44% of receptors possess high affinity with dissociation constant for carbachol, $K_H = 2.8 \times 10^{-6}$ M, dissociation constant of low-affinity receptor, $K_L = 2.3 \times 10^{-6}$ M; forebrain: 41% high affinity, $K_H = 2.1 \times 10^{-7}$ M, $K_H = 1.7 \times 10^{-5}$ M. The data suggest that whole brain contains at least three major muscarinic receptors which can be distinguished on the basis of their affinities for agonists.

Ellis. J. and *Hoss, W.*

Brain Research 193:189-198, 1980.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY.

COMPETITIVE INTERACTION OF GALLAMINE WITH MULTIPLE MUSCARINIC RECEPTORS

Neural membranes from the brains of male Sprague-Dawley rats were prepared and used for binding studies with gallamine and carbachol. In the paper presented here, it is shown that gallamine, a nicotinic antagonist with antimuscarinic potency in several systems, interacts competitively with the tritiated ligand [^{3}H]quinuclidinyl benzilate (QNB) at the muscarinic receptor. The occupancy curves derived from these studies suggest that gallamine has widely varying affinities for different subpopulations of muscarinic receptors, a finding which sets gallamine apart from classical muscarinic antagonists such as atropine and QNB. The greatest difference in affinities for gallamine occurred in the brain stem, where the data could be satisfactorily fitted to a two-site model, with 77% of the receptors having high affinity (K_d = 25 nM) and 23% low affinity (93 μM). Further, these affinities displayed rank order correlating with those of carbachol (an agonist), although gallamine has not, so far, displayed agonist (or partial agonist) activity. The finding that antagonists as well as agonists can display multiple affinities for muscarinic receptors suggests that there are fundamental differences among subpopulations of these receptors.

Ellis, J. and *Hoss, W.*

Biochemical Pharmacology 31(5):873-876, 1982.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY.

100231708

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

V. *Pharmacology and Biochemistry*

AN ISOLATED PERFUSED DOG-LUNG PREPARATION FOR THE STUDY OF CYCLIC GMP METABOLISM: EFFECTS OF SODIUM NITROPRUSSIDE AND OXYGEN

In recent years, considerable interest has developed in the role that cyclic GMP may play in pulmonary function, partly because the lung has a high guanylate cyclase activity compared with other tissues and partly because the lung is in direct contact with various environmental pollutants, many of which are known to precipitate the formation of oxygen free radicals and damage the lung. For the study presented here, the intact, isolated perfused dog lung was evaluated as a model for studies directed at defining the role of oxidative modulation of lung cyclic GMP metabolism in pulmonary function. Sodium nitroprusside added to the perfusion blood increased the cyclic GMP content of lung over four-fold in a dose-dependent manner. Although sodium nitroprusside administration caused changes in lung vascular resistance, these occurred independently of the changes in cyclic GMP. Ventilation of lungs with a high oxygen gas mixture containing 95% 0_2, 5% CO_2 acutely increased the cyclic GMP content of lungs after 15 minutes from 1.3 ±0.06 to 3.4 ±0.12 pmol cyclic GMP/mg protein. Cyclic GMP levels returned toward control during continued ventilation with the high oxygen concentration. The oxygen-induced elevation of lung cyclic GMP content was not accompanied by changes in lung vascular resistance. The results indicate that the isolated perfused lung may be useful in studies of cyclic GMP, tissue oxidation and pulmonary function.

Braughler, J. M. and Maron, M. B.

European Journal of Pharmacology 78:187-193, 1982.

Other support: American Lung Association.

From the Programs in Pharmacology and Physiology, Northeastern Ohio Universities College of Medicine, Rootstown.

INVOLVEMENT OF SULFHYDRYL GROUPS IN THE OXIDATIVE MODULATION OF PARTICULATE LUNG GUANYLATE CYCLASE BY NITRIC OXIDE AND N-METHYL-N-NITRO-NITROSOGUANIDINE

This report describes the activation of particulate rat lung guanylate cyclase by nitric oxide and N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and suggests the involvement of sulfhydryl groups in the activation process. In this study, particulate guanylate cyclase from rat lung was activated by nitric oxide or MNNG in a dose-dependent manner that was affected by dithiothreitol. Although low concentrations of nitric oxide or MNNG produced near maximum activation, excessive amounts decreased the particulate enzyme activity. Nitric oxide-stimulated guanylate cyclase activity decayed during a 60-minute preincubation period at 37°C, but did not decay at 24° or 4°. Dithiothreitol enhanced the decay of nitric oxide-stimulated enzyme at all temperatures by potentiating the reversal of nitric oxide activation. Following the reversal of nitric oxide activation at 24° by dithiothreitol, the particulate enzyme could

10023170B2

Source: https://www.industrydocuments.ucsf.edu/docs/fqhk0000

be reactivated by a second exposure to nitric oxide. Preincubation of basal particulate guanylate cyclase activity at 37° resulted in the loss of enzyme responsiveness to activation by nitric oxide or MNNG. This loss of responsiveness that was prevented by the thiol antioxidants was potentiated by the thiol oxidants, diamide or oxidized glutathione. Also, the inhibitory effects of the thiol oxidants on enzyme responsiveness to activation by MNNG were prevented by dithiothreitol. These results suggest that the activation of particulate guanylate cyclase by nitric oxide or MNNG involves the oxidation of key enzyme sulfhydryl groups.

Braughler, J. M.

Biochemical Pharmacology 31(7):1239-1244, 1982.

From the Program in Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown.

SEPARATION AND DETECTION OF LIPOPROTEINS IN HUMAN SERUM BY USE OF SIZE-EXCLUSION LIQUID CHROMATOGRAPHY: A PRELIMINARY REPORT

This methodological paper presents a relatively mild and quite rapid procedure for separating serum lipoproteins for individual collection. In a first step, human serum components, including lipoproteins, can be rapidly separated by size-exclusion "high-performance" liquid chromatography. Then, lipoproteins in fractions of the eluate can be quantitated by conventional chemical and enzymatic methods. Alternatively, if lipoproteins in the serum are selectively prestained with diformazan dye, the column effluent can be monitored spectrophotometrically at 580 nm, so that only the lipoprotein components of serum are detected. Samples of purified low-density lipoproteins, so stained and analyzed, provide peak-area values that are proportional to their concentration as evaluated by chemical methods. With this technique, the various lipoprotein classes can be quickly separated and their concentration estimated. Overall, these techniques seem to have potential for development into analytical and clinical procedures.

Busbee, D. L. et al.

Clinical Chemistry 27(12):2052-2058, 1981.

Other support: American Cancer Society, Robert A. Welch Foundation and a North Texas State University Faculty Research Grant.

From the Departments of Biological Sciences and Chemistry, North Texas State University, Denton, and the Department of Biochemistry, Texas College of Osteopathic Medicine, Ft. Worth.

A NEW SUBCUTANEOUSLY-IMPLANTABLE RESERVOIR FOR SUSTAINED RELEASE OF NICOTINE IN THE RAT

Nicotine has become a widely studied drug for its pharmacological and toxicological effects. In previous animal studies, forced-administration methods for nicotine included parenteral injection, administration by inhalation of cigarette smoke, solubili-

1002317083

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

zation in drinking water, and injection in single doses or chronically via Alzet minipump into the ventricles of the brain, among others. This paper now reports the successful development of a subcutaneously-implantable reservoir for the sustained release of nicotine. The device, dubbed INR for Implantable Nicotine Reservoir, is a small glass cup sealed with Silastic® polymer. It releases 3.4 mg of nicotine per 24 hours. When implanted into moderately-sized female Sprague-Dawley rats it produces blood nicotine levels of 400-500 ng/ml which remain relatively stable over at least 18 days. INRs are nontoxic, reproducible, inexpensive, and adaptable for pharmacological and toxicological studies in rats and other small animals.

Erickson, C. K. et al.

Pharmacology Biochemistry & Behavior 17:183-185, 1982.

From Drug Dynamics Institute, College of Pharmacy, The University of Texas, Austin.

DETERMINATION OF THE PRIMARY METABOLITE OF CENTRAL NERVOUS SYSTEM NOREPINEPHRINE, 3-METHOXY-4-HYDROXYPHENETHYLENEGLYCOL, IN MOUSE BRAIN AND BRAIN PERFUSATE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

The rate of production of 3-methoxy-4-hydroxyphenethyleneglycol (MHPG), the principal metabolite of norepinephrine in mamalian brain, has been proposed to be an indicator of the rate of norepinephrine turnover. For this reason, new methods of MHPG isolation and detection are important in furthering man's knowledge of the role of norepinephrine in normal and dysfunctional mamalian brain. In the present methodology paper, assays are described for the determination of picomole levels of MHPG in mouse brain and in the perfusate of an intact mouse brain. High-performance liquid chromatography with electrochemical detection yielded a MHPG detection limit of 0.37 pmol. This technique offers a sensitive and inexpensive alternative to gas chromatography with mass spectrometry, and can be used in conjunction with brain catecholamine determinations.

Towell, J. F. and *Erwin, V. G.*

Journal of Chromatography 223:295-303, 1981.

Other support: U. S. Public Health Service.

From the School of Pharmacy, University of Colorado, Boulder.

PRIMARY STRUCTURE OF THE SIGNAL PEPTIDE OF TROPOELASTIN b

Elastin is a major structural component of connective tissues. Its soluble precursor, tropoelastin, is extractable in organic solvents and possesses an extensive clustering of nonpolar amino acid residues in the immediate NH_2-terminal region. Since many proteins secreted from eukaryotic cells are initially synthesized with signal peptides, tropoelastin was examined to see if it did in fact contain a signal peptide. Leader sequences, or signal peptides, are relatively short hydrophobic NH_2-terminal exten-

1002317084

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

sions, which are thought to play a role in vectorial transport of the nascent polypeptide. Therefore, the possibility that the initial tropoelastin translation product possesses a short signal peptide was examined in a cellfree translation system. In this study, total RNA, isolated from aortae of 1-day-old chicks, was translated in an mRNA-dependent reticulocyte lysate translation assay. The translation products were then immunoprecipitated and subjected to automated radio-sequencing. Comparison of the NH_2-terminal sequence of tropoelastin b synthesized in the cell-free system versus that synthesized in organ culture demonstrated the presence of a signal peptide 24 amino acid residues in length. The signal peptide sequence is as follows: Met-Arg-Gln-Ala-Ala-Ala-Pro-Leu-Leu-Pro-Gly-Val-Leu-Leu-Leu-Phe-Ser-Ile-Leu-Pro-Ala-Ser-Gln-Gln. The preponderance of hydrophobic amino acid residues as well as the polar residues adjacent to the initiator methionine and the carboxyl termini found in the signal peptide is similar to that reported for other secreted proteins.

Karr, S. R. and *Foster, J. A.*

The Journal of Biological Chemistry 256(12):5946-5949, 1981.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

ABSENCE OF SEASONAL VARIATION IN ANTIPYRINE METABOLISM

Aryl hydrocarbon hydroxylase (AHH), the monooxygenase system(s) that metabolizes benzo(α)pyrene to fluorescent phenols, has been shown to exhibit a strong seasonal variation. The *in vivo* metabolism of antipyrine, which is also catalyzed by microsomal cytochrome P-450-dependent monooxygenases, has been reported to be correlated with AHH inducibility in human lymphocytes. In the study presented here, an attempt was made to determine whether antipyrine metabolism, like AHH metabolism, shows seasonal changes. To do this, antipyrine half-life (t1/2) was measured in 10 nonsmokers and eight smokers in the summer and the winter, the two times of the year that correspond to the high and low peaks of inducible AHH activity as measured in lymphocytes. Results showed that the mean antipyrine t1/2 determined in all 18 subjects in summer was almost identical to that found in winter ($x \pm SEM = 10.90 \pm 0.65$ and 10.96 ± 0.78 hr). AHH activity in cultured human lymphocytes from the nonsmoking subjects was determined in control and 3-methylcholanthrene-induced cells to obtain inducibility ratios of 4.2 ± 0.56 (SEM) in the summer and 1.4 ± 0.14 (SEM) in the winter. These results indicate that the seasonal variation in AHH inducibility in human lymphocytes is not reflected by a corresponding seasonal variation in antipyrine metabolism *in vivo*.

Paigen, B., Ward, E., Steenland, K., Bolanowska, W., *Gessner, T.*, Chang, R. L., Wood, A. W., and Conney, A. H.

Clinical Pharmacology & Therapeutics 31(2):144-150, 1982.

Other support: National Cancer Institute.

From the Departments of Molecular Biology and Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, and the Department of Biochemistry and Drug Metabolism, Hoffmann-LaRoche Inc., Nutley, NJ.

1002317085

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

ENZYMATIC PROPERTIES OF HUMAN GLUCURONYLTRANSFERASE AND A SENSITIVE METHOD FOR ITS ASSAY IN A STABLE B LYMPHOCYTE CELL LINE

UDP-glucuronyltransferases are membrane-bound enzymes located chiefly in the endoplasmic reticulum of cells and are responsible for the conjugation of endogenous and xenobiotic substances containing hydroxyl, amino, thiol, or carboxylic functional groups. Although there is much interest in human glucuronyltransferases, extensive studies of them have been hampered by difficulties associated with procurement of sufficient quantities of fresh human cells. This report, however, demonstrates that a prolific stable cell line, such as SN1006, capable of yielding billions of cells could be used for such studies. Homogenates of SN1006 cells have been studied. A sensitive assay procedure for lymphocyte glucuronyltransferase was developed utilizing radioactive testosterone as the acceptor substance and TLC for separation of the metabolite. The method is capable of detecting picomolar quantities of the product. The enzyme activity exhibited a broad pH optimum, and was subject to activation by the detergent Lubrol WX and Mn^{++} ions. The activity conformed to the Michaelis-Menten kinetics giving apparent K_m values of 0.8 mM and 11 μM, for UDPGA and testosterone, respectively. 4-Methylumbelliferone, α-naphthol and *P*-nitrophenol behaved as competitive inhibitors of testosterone glucuronidation. The results presented in this paper indicate that the method could be used for genetic studies of human lymphocyte glucuronyltransferase, and that the enzyme is of consequence in detoxication of exogenous as well as endogenous substrates.

Li, H. C., Porter, N. and *Gessner, T.*

Enzyme 28:54-65, 1982.

Other support: U. S. Public Health Service and the New York State Department of Health.

From the Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo.

SUBSTRATE SPECIFICITY OF HUMAN UDP-GLUCURONYLTRANSFERASE IN CULTURED LYMPHOCYTES

Human lymphocytes possess glucuronyltransferase activity with a broad range of substrate specificity. For the paper presented here, catalytic properties of lymphocyte glucuronyltransferase were studied, and activity for the following substances was documented: testosterone, estradiol, phenolphthalein, α-naphthol, 4-methylumbelliferone and *p*-nitrophenol. As has been noted before, α-naphthol is regarded as a model aglycone for the form of glucuronyltransferase which catalyses glucuronidation of the less bulky nonsteroidal substrates, and testosterone as that for the steroidal aglycones. The results show that human lymphocytes possess glucuronyltransferase activity for both types of aglycone. Within limitations of work with crude homogenates and 60 minute incubations, no difference could be seen in apparent K_m values of UDPGA

10023170B6

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

when the various aglycone substrates were tested. Furthermore, competitive inhibition of testosterone UDP-glucuronyltransferase was observed when 4-methylumbelliferone, α-naphthol and *p*-nitrophenol were used as the inhibitors. These data demonstrate the similarity of the enzyme(s) accepting the various substrates.

Li, H. C., Porter, N., Holmes, G., and *Gessner, T.*

Xenobiotica 11(10):647-654, 1981.

Other support: New York State Department of Health.

From the Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo.

STUDIES ON THE DEPOSITION AND DISTRIBUTION OF CATECHOL FROM WHOLE CIGARETTE SMOKE IN BCF1/CUM MICE

The studies reported here were performed to determine the deposition, distribution, and clearance of catechol in cigarette smoke using defined smoke exposure conditions and a well-characterized mouse strain (BC3F1/Cum). The presence of [^{3}H]catechol in the smoke was verified by silica gel chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometry. Mice were exposed to 10%(v/v)2R1 cigarette smoke on the Walton Horizontal Smoking Machine under standard conditions of 35 ml puff volume, 2 sec/puff, 10 puffs/cigarette. The deposition and distribution of inhaled catechol were determined in all internal tissues, urine, and feces. Data showed that clearance was occurring during the 10-min smoke exposure period. Immediately after exposure, over 50% of the radioactivity was found in the blood, with 10% found in the lung, and approximately 12% in the respiratory tract. Over 91% of the inhaled radioactivity was found in the urine 120 min after exposure. Less than 0.5% of the total dose was found in the lung at this time. In summary, these determinations show that catechol in smoke is rapidly absorbed, redistributed and excreted from mice exposed to whole cigarette smoke.

Hwang, K. K., Sonko, O., Dansie, D. R., Kouri, R. E., and Henry, C. J. *(Microbiological Associates)*

Toxicology and Applied Pharmacology 64(3):405-414, 1982.

From the Department of Experimental Oncology, Microbiological Associates, Bethesda, MD.

EFFECTS OF NICOTINE ON UTERINE BLOOD FLOW AND INTRAUTERINE OXYGEN TENSION IN THE RAT

Since it was known that normal conceptus development requires optimal levels of oxygen, it seemed reasonable to determine whether nicotine-induced reduction in uterine blood flow concomitantly decreases the concentration of oxygen within the uterine lumen. In the study presented here, it was seen that subcutaneous injection of

1002317087

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

nicotine (0.5 or 5 mg/kg body wt) resulted in a marked and prolonged reduction in uterine blood flow and intrauterine oxygen tension in pseudopregnant rats (Day 4). By 10 minutes after nicotine administration (5 mg/kg) uterine perfusion was reduced by 40%, remained suppressed for 90 minutes and returned to the pre-treatment level by 120 minutes. Rats receiving the 0.5 mg nicotine/kg also showed a marked reduction in uterine blood flow, although the response was slower in onset and longer in duration. Nicotine (5 mg/kg), also resulted in a sustained decrease in intrauterine oxygen tension from a control value of 48.9±3.6 to 22.2±2.6 mmHg at 45-60 minutes and 21.7±1.5 mmHg at 60-90 minutes. The frequency and amplitude of fluctuations in intrauterine oxygen tension were still reduced by 90 minutes after treatment. Therefore, the results of this study indicate that nicotine, in amounts sufficient to suppress embryonic growth, reduces uterine blood flow and produces a marked and sustained decrease in oxygen tension within the uterine lumen. The time-course of the reduction in intrauterine oxygen availability parallels that of the nicotine-induced decrease in uterine blood flow.

Hammer, R. E., Goldman, H. and *Mitchell, J. A.*

Journal of Reproduction and Fertility 63:163-168, 1981.

From the Departments of Anatomy and Pharmacology, Wayne State University School of Medicine, Detroit.

SYNTHESIS OF NON-K-REGION *ORTHO*-QUINONES OF POLYCYCLIC AROMATIC HYDROCARBONS FROM CYCLIC KETONES

In this methodological paper, non-K-region *o*-quinones of polycyclic aromatic hydrocarbons are prepared easily by heating a solution of a tetrahydrodiol with 12 equiv. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane (reflux, 24 hrs). DDQ not only oxidizes the 1,2-diol moiety α-diketone but also introduces the olefinic double bond of the *o*-quinone. When this one-step conversion was first discovered, the surprisingly facile generation of the *o*-quinone was attributed to the ease of double bond formation at the bay-region as described for several tetrahydro arenes. However, present results indicate that the reaction is not restricted to the synthesis of non-bay-region *o*-quinones but is also applicable to the preparation of bay-region *o*-quinones. Thus, this method is both simpler and more general than the recently published synthesis of non-K-region *o*-quinones. Overall, the synthetic approach presented here constitutes a new nethod for the preparation of non-K-region *o*-quinones that seems to be generally applicable.

Platt, K. L and *Oesch, F.*

Tetrahedron Letters 23(2):163-166, 1982.

From the Department of Toxicology, Institute of Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

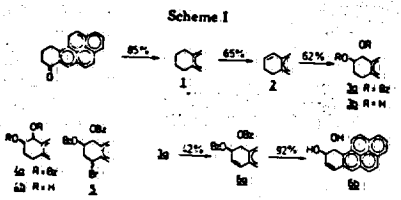
1002317088

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

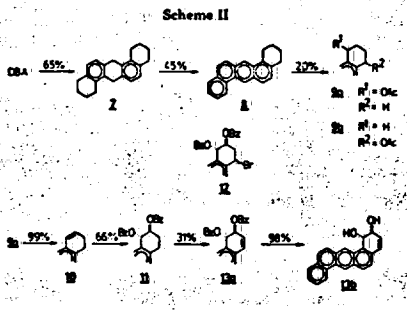
IMPROVED SYNTHESIS OF (±)-TRANS-9-10-DIHYDROXY-9-10-DIHYDRO-BENZO[α]-PYRENE AND OF (±)-TRANS-1-2,-DIHYDROXY-1,2-DIHYDRO-DIBENZ[*a,h*]ANTHRACENE

Non-K-region dihydrodiols of polycyclic aromatic hydrocarbons (PAH) play an important role in the metabolism of PAH. They are precursors of dihydrodiol epoxides, some of which are considered ultimate carcinogenic metabolites of PAH. While the non-K-region dihydrodiols with the olefinic bond in the bay region have been intensively studied, the biological role of the non-K-region dihydrodiols of benzo(α)pyrene and dibenz[*a,h*] anthracene (6b and 13b), where the olefinic bond is not of the bay region, is less well known. In the present study, improved synthetic pathways for 6b and 13b were devised that resulted in the schemes seen below: a six-step synthesis of 6b (Scheme I) and eight-step synthesis of 13b (Scheme II).

Scheme I



Scheme II



Since the little interest in the dihydrodiols 6b and 13b stems partly from the fact that their syntheses described in the literature are more difficult than those of the better studied isomers, it is hoped that these new improved syntheses may help in furthering study of the biological roles of 6b and 13b.

Oesch, F. et al.

The Journal of Organic Chemistry 47:568-571, 1982.

From the Institute of Pharmacology, Section of Biochemical Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

K-REGION *TRANS*-DIHYDRODIOLS OF POLYCYCLIC ARENES; AN EFFICIENT AND CONVENIENT PREPARATION FROM *O*-QUINONES OR *O*-DIPHENOLS BY REDUCTION WITH SODIUM BOROHYDRIDE IN THE PRESENCE OF OXYGEN

K-region *trans*-dihydrodiols are important metabolites of polycyclic aromatic hydrocarbons. They are produced by epoxide hydrolase-mediated hydration of the primarily formed arene oxides. The K-region *trans*-dihydrodiols are usually synthesized by stereoselective reduction of the corresponding *o*-quinones with complex metal hydrides such as lithium aluminum hydride. When first attempts were made to use sodium or potassium borohydride in methanol or ethanol for the reduction of K-region *o*-quinones, they were not very successful. Also, the use of different reaction times and temperatures, different ratios of the reactants, and different solvents did not improve

1002317089

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

the results substantially. However, it was finally found that polycyclic *o*-quinones can be conveniently reduced to the corresponding *trans*-dihydrodiols by carrying out the reduction with borohydrides in the presence of oxygen. The role of oxygen in the reduction may be rationalized by a mechanism presented in this paper. As a result of the reduction-oxidation cycle that takes place when oxygen is available, pure *trans*-dihydrodiol accumulates so that it can be isolated in high yield. This new method of reducing K-region *o*-quinones (and also the corresponding *o*-diphenols and their acetates) derived from polycyclic arenes represents a significant improvement of the synthesis of K-region *trans*-dihydrodiols.

Platt, K. L. and *Oesch, F.*

SYNTHESIS: International Journal of Methods in Synthetic Organic Chemistry No. 6:459-462, June 1982.

From the Institute of Pharmacology, Section on Biochemical Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

ANALYSIS OF NICOTINE AND COTININE IN TISSUES BY CAPILLARY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY—MASS SPECTROMETRY

In this methodological paper, the development and application of methods to quantitate low levels of nicotine and cotinine in tissue samples are described. Analyses were performed by capillary column gas chromatography with a specific nitrogen-phosphorus detector and by gas chromatography-mass spectrometry. With close structural analogs for internal standards, high quantitative accuracy and precision were demonstrated for the range of 5-1000 ng per g of tissue. The sensitivity limit was 2-3 ng/g for both compounds. The main advantage of these techniques compared to previously published methods is increased selectivity; the other methods were developed for analysis of biological fluids and are not readily adaptable to more complex biological matrices such as tissue homogenates. With the newly developed techniques, it was possible to perform a pharmacokinetic study of nicotine and cotinine in mouse liver following a single intraperitoneal injection of nicotine.

Thompson, J. A., Ho, M-S. and *Petersen, O. R.*

Journal of Chromatography 231:53-62, 1982.

From the School of Pharmacology, University of Colorado, Boulder.

RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND CHOLINERGIC ACTIVITIES OF FURMETHIDES AND POTENTIATION OF THESE ACTIVITIES BY PHYSOSTIGMINE

The furmethide compounds–furfuryltrimethylammonium (furmethide; FT), 5-methylfurmethide (5-MFT), 5-hydroxymethylfurmethide (5-HMFT) and 5-chloromethylfurmethide (5-ClMFT)–are potent and stable muscarinic agents which are being used in pharmacological investigations where muscarinic selectivity is required. For the studies reported here, the cholinergic activities of the four furmethides were compared on a superfused guinea pig ileum preparation. Results showed that all four components were less potent but equally as active as acetylcholine (ACh). The ED50s

1002317090

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

of these compounds decrease in the following order: 5-C1MFT > 5-HMFT > FT > 5-MFT. The electron densities around the furan-oxygen atoms of these compounds decrease in the same order. A careful analysis of the effects of these agents on the guinea pig ileum revealed that the furmethides display two separate actions: (1) direct interaction at the muscarinic receptor to elicit a response, and (2) release of ACh from the presynaptic nerve terminal. In the presence of physostigmine, dose response curves of these compounds were shifted to the left. There was approximately a two-fold shift in the ED50 values for the furmethide. Like muscarine, these compounds were not hydrolyzed by cholinesterase (ChE). At least part of the noted furmethides response was due to release of ACh, because the responses were increased by ChE inhibition.

Chaturvedi, A. K., Rowell, P. P., and *Sastry, B. V. R.*

Pharmacological Research Communications 13(9):829-845, 1981.

Other support: National Institute of Child Health and Human Development.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

CHANGES IN MICROSOMAL MEMBRANE MICROVISCOSITY AND PHOSPHOLIPID METHYLTRANSFERASES DURING RAT LIVER REGENERATION

Regenerating liver is a good example of a controlled growing tissue. During liver growth, newly synthesized molecules of drug-metabolizing enzymes like cytochrome P450 are incorporated into the lipid matrix of endoplasmic reticulum (ER). There is a morphological association of ribosomal protein synthesis with ER membranes. Recent research has shown that decreased membrane microviscosity facilitates incorporation of the enzyme-protein molecules into the phospholipid matrix of ER. The microviscosity of membranes is altered by: (1) changed ratios of phospholipid to cholesterol, (2) unsaturation of fatty acids in phospholipids, and (3) methylation of phospholipids. In a related study, microviscosity and S-adenosyl-L-methionine (SAM) mediated methylation of phosphatidylethanolamine to phosphatidyl-N-methylethanolamine (PME) and phosphatidylcholine (PC) were measured using microsomal membranes of regenerating rat livers at 6-96 hrs after partial hepatectomy. In the methylated phospholipids, the proportion of PME increased by 3-9% at 1 μM SAM, and, at 200 μM SAM, the proportion of PC decreased by about 5-10% at 12-24 hrs. Two phase transitions were observed with microsomal membranes between 20 and 40°C. In synthetic liposomes containing PE, PME and PC, microviscosity decreased when the proportion of PME increased or the proportion of PC decreased. Therefore, alterations in phospholipid methyltransferases and consequent changes in membrane phospholipid methylation may contribute to increased membrane fluidity during cell proliferation and incorporation of drug-metabolizing enzymes into ER.

Jaiswal, R. K., *Sastry, B. V. R.* and Landon, E. J.

Pharmacology 24:355-365, 1982.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

100231709

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

ENHANCEMENT OF THE RESPONSIVENESS OF THE RAT DIAPHRAGM BY L-METHIONINE AND PHOSPHOLIPID METHYLATION AND THEIR RELATIONSHIP TO AGING

The contractions of hemidiaphragms of Fischer 344 rats of age 2 to 33 months were measured after electrical stimulation of the phrenic nerve or the muscle *in vitro*. Earlier work has shown that S-adenosyl-L-methionine (SAM)-mediated methylation of membrane phospholipids increases membrane fluidity and responsiveness of the muscle, and that an increase in the intracellular levels of S-adenosyl-L-homocysteine (SAH) inhibits phospholipid methylation. In the study presented here, the intracellular levels of SAM were increased by incubating the hemidiaphragm in L-methionine, L-homocysteine thiolactone, adenosine and erythro-9-(2-hydroxy-3-nonyl) adenine, an inhibitor of adenosine deaminase. The following results were obtained: (1) Microsomes from hemidiaphragm contained phospholipid methyltransferases, (2) L-methionine increased muscle tension developed by electrical stimulation of the muscle or the nerve. This increase in the tension is dependent upon the concentration of L-methionine. (3) Labeled methyl groups were incorporated from labeled L-methionine into phospholipids of the hemidiaphragm. (4) Presence of adenosine, L-homocysteine thiolactone and erythro-9-(2-hydroxy-3-nonyl)adenine inhibited the effect of L-methionine to increase muscle tension and incorporation of methyl groups into phospholipids. (5) Muscle tension developed by electrical stimulation of the phrenic nerve or the muscle decreased with increasing age, and (6) The methionine effect was erratic or insignificant in hemidiaphragms of old rats (>15 months). These observations indicate that increasing the cellular formation of SAM increases phospholipid methylation and the contraction heights of the hemidiaphragm upon electrical stimulation. Both of these effects are inhibited by increasing levels of SAH. They also indicate that SAM-mediated membrane phospholipid methylation and fine regulation of membrane fluidity in the diaphragm may become defective with advancing age, and may contribute partly to the functional deficits of the diaphragm.

Sastry, B. V. R., Owens, L. K. and Janson, V. E.

The Journal of Pharmacology and Experimental Therapeutics 221(3):629-636, 1982.

Other support: U.S. Public Health Service, National Institute of Aging, and the National Institute of Child Health and Human Development.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REGULATION OF ACETYLCHOLINE RELEASE IN THE MOUSE CEREBRUM BY METHIONINE ENKEPHALIN AND SUBSTANCE P

Methionine enkephalin (MEK) and Substance P (SP) may regulate acetylcholine (ACh) release in the cerebrum. In the present attempt to study this process, mouse cerebral slices were incubated in a modified Krebs bicarbonate buffer containing (methyl-^{3}H) choline chloride. The slices were filtered, washed and transferred to a microbath set up for superfusion. ACh release was followed by the efflux of labeled

1002317092

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

ACh and choline as a function of time. The release of opioid peptides was measured by specific radioimmunoassays. The rate of ^{3}H-ACh release increased initially for the first five min., reached a peak, and then declined exponentially (half-time, 35 min.). Electrical field stimulation of the slices during the exponential phase caused a significant increase in ACh release for five min. Absence of exogeneous Ca^{++} in the perfusion field depressed both spontaneous and evoked release of ACh. When the effects of long acting enkephalin (D-ala-enkephalinamide, DALA) and SP on ACh release were studied, DALA (34 nM) decreased the release of ACh (65%) as well as Ca^{++} uptake (48%). SP(6×10^{-7} M) increased ACh release (35%) and increased Ca^{++} uptake (154%). These observations indicate that enkephalins may exert a negative feedback control of ACh release from cerebral slices by inhibiting Ca^{++} uptake by the slices. Similarly, SP may exert a positive feedback on the ACh release by enhancing the uptake of Ca^{++} by cerebral slices.

Sastry, B. V. R. and Tayeb, O. S.

In: Dhawan, B. N. (ed.): *Current Status of Centrally Acting Peptides*, New York: Pergamon Press, 1982, pp. 165-172. (also in: *Advances in the Biosciences:* 165-172, 1982.)

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

ION BEAM TRITIUM LABELING OF PROTEINS AND PEPTIDES

An advanced technique for tritiating proteins that yields high specific radioactivities without causing significant changes in biological activities is presented here. In this method, a carefully controlled particle beam composed of T_3^+ and T_2^+ ions and fast T_2 molecules is accelerated into a sample target within a vacuum chamber. This beam method has been used to tritiate ribonuclease A, porcine pancreatic elastase, thermolysin, soybean trypsin inhibitor, α_1-protease inhibitor, and the peptide aldehydes leupeptin and antipain. After removal of all readily exchangeable tritium, the products were obtained in 32-83% yields with specific radioactivities of 18-856 Ci/mol. The products were carefully characterized, shown to be chemically pure, and to have complete biological activity. Simple tritium hydrogen exchange accounts for at least 82% of the reaction pathway with proteins and for 100% of the reaction with the peptide aldehydes. This method is practical and economical on a reasonable scale, producing high specific tritium incorporation without significant chemical decomposition or structural change to even fragile, high molecular weight materials.

Bush, G. A. *et al. (Travis, J.)*

The Journal of Biological Chemistry 256(23):12213-12221, 1981.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

10023170 93

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

ISOLATION AND PROPERTIES OF HUMAN NEUTROPHIL MYELOPEROXIDASE

Purification of human leukocyte myeloperoxidase, as described in this paper, is a simple three-step procedure involving dialysis of a granule extract against low-salt buffer, Sephadex G-75 chromatography, and carboxymethylcellulose chromatography. The final product of this purification is homogeneous when examined by acid polyacrylamide gel electrophoresis and sedimentation equilibrium ultracentrifugation. The molecular weight that was determined here by the latter procedure was 118,000. With or without reduction of the protein by 2-mercaptoethanol, subunits were formed which migrated as a single band after sodium dodecyl sulfate gel electrophoresis. The molecular weight of the apparently identical subunits was 59,000 with reduction, and 42,000 without reduction. Other general properties of human leukocyte myeloperoxidase, including amino acid composition, amino terminal sequence analysis, and absorption spectra, are also reported. Myeloperoxidase, in the presence of hydrogen peroxide and chloride ion, and no other substrate, autoinactivates. After completion of the inactivation reaction, several oxidizable amino acids in the enzyme are modified, and the absorption peak at 430 nm disappears. The presence of substrate of the myeloperoxidase system (α-1-proteinase inhibitor), or of high concentration of chloride ion, completely protects the enzyme from autoinactivation.

Matheson, N. R., Wong, P. S. and *Travis, J.*

Biochemistry 20(2):325-330, 1981.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

INTERACTION OF HUMAN α_1-PROTEINASE INHIBITOR WITH NEUTROPHIL MYELOPEROXIDASE

In earlier studies, it has been shown that α-1-proteinase inhibitor (α-1-PI) can be inactivated by myeloperoxidase in the presence of hydrogen peroxide and chloride ion. Since myeloperoxidase may be readily released from neutrophils, its oxidative inactivation of α-1-PI may indirectly result in enhancement of proteolytic destruction of lung tissue, even in individuals with genetically normal levels of inhibition. The aim of this investigation, therefore, was to further investigate the parameters of the myeloperoxide-mediated oxidative inactivation of α-1-PI. These studies, as reported here, demonstrate that there is a direct dependence on the concentration of α-1-PI which becomes saturating at a concentration of about 4.5 μM. There is also a dependence on the concentration of H_2O_2 to 95 μM, after which increasing concentrations become increasingly inhibitory. Chloride ion is required for myeloperoxidase oxidative action, but the cations Na^+, NH_4^+, or K^+ have little effect. There is a very sharp pH optimum at pH 6.2, under physiological NaCl concentration, and approximately half the rate of inactivation of α-1-PI occurs at pH 5.9 or 6.5. Sodium dodecyl sulfate gel electrophoresis indicates that α-1-PI oxidized by either myeloperoxidase or N-chlorosuccinimide is not different in size from native α-1-PI. When oxidized α-1-PI is incubated with porcine elastase, the α-1-PI is converted to a modified form of lower molecular weight. Amino acid sequence analysis confirms these results in that the sequence is different from that of native α-1-PI. Overall, from this data and other observations reported here, it is apparent that oxidative processes directly affect the inhibitory activity of α-1-PI modification of the reactive-site methionine of this protein.

1002317094

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000

Matheson, N. R., Wong P. S., Schuyler, M., and *Travis, J.*

Biochemistry 20(2):331-336, 1981.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

INACTIVATION OF HUMAN PLASMA α_1-PROTEINASE INHIBITOR BY A METALLOPROTEINASE FROM *SERRATIA MARCESCENS*

This study was undertaken to investigate the interaction of human plasma α_1-proteinase inhibitor with a metalloproteinase isolated from *Serratia marcescens*. Results presented here show that the interaction of α_1-proteinase inhibitor with the *Serratia* proteinase caused a rapid decrease in inhibitory activity towards trypsin which was both concentration- and time-dependent. To determine whether the inactivation had occurred by limited proteolysis, proteinase-α_1-proteinase inhibitor incubation mixtures were denatured and analyzed by SDS-polyacrylamide gel electrophoresis. These results show that the proteinase had rapidly converted α_1-proteinase inhibitor into an inactive form of lower molecular weight (48,000 for the modified form vs. 52,000 for the native inhibitor). Amino terminal sequence analysis indicated that the interaction of the inhibitor and enzyme was at the reactive site of the inhibitor, with peptide-bond cleavage resulting in the inactivation. This process may be important in necrotic processes occurring during bacterial infiltration of host tissues.

Virca, G. D., Lyerly, D., Kreger, A., and *Travis, J.*

Biochimica et Biophysica Acta 704:267-271, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens, and the Department of Microbiology and Immunology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC.

VI. *Immunology and Adaptive Mechanisms*

CHEMOTACTIC ACTIVITY GENERATED FROM THE FIFTH COMPONENT OF COMPLEMENT BY PLASMA KALLIKREIN OF THE RABBIT

Kallikrein purified from rabbit plasma has been shown in the study presented here to generate chemotactic activity for rabbit neutrophils from the rabbit's fifth component of complement (C5). The effect on C5 appeared to be due to kallikrein itself and not to a contaminating enzyme, because it could be inhibited by anti-kallikrein IgG or by soybean trypsin inhibitor to the same extent that kinin generation by the same

10023170 95

Source: https://www.industrydocuments.ucsf.edu/docs/rqhk0000